Relationship Among Plasma Cortisol, Catecholamines, Neuropeptide Y, and Human Performance During Exposure to Uncontrollable Stress

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Objective: Although many people are exposed to trauma, only some individuals develop posttraumatic stress disorder; most do not. It is possible that humans differ in the degree to which stress induces neurobiological perturbations of their threat response systems, which may result in a differential capacity to cope with aversive experiences. This study explored the idea that differences in the neurobiological responses of individuals exposed to threat are significantly related to psychological and behavioral indices. **Methods:** Individual differences in neurohormonal, psychological, and performance indices among 44 healthy subjects enrolled in US Army survival school were investigated. Subjects were examined before, during, and after exposure to uncontrollable stress. **Results:** Stress-induced release of cortisol, neuropeptide Y, and norepinephrine were positively correlated; cortisol release during stress accounted for 42% of the variance in neuropeptide Y release during stress. Cortisol also accounted for 22% of the variance in psychological symptoms of dissociation and 31% of the variance in military performance during stress. **Conclusions:** Because dissociation, abnormalities in the hypothalamic-pituitary-adrenocortical axis, and catecholamine functioning have all been implicated in the development of stress disorders such as posttraumatic stress disorder, these data suggest that some biological differences may exist *before* index trauma exposure and *before* the development of stress-related illness. The data also imply a relationship among specific neurobiological factors and psychological dissociation. In addition, the data provide clues about the way in which individuals' psychobiological responses to threat differ from one another. **Key words:** neuropeptide Y, cortisol, stress, military, performance, dissociation.

ANOVA = analysis of variance; CADSS = Clinician-Administered Dissociative Symptom Scale; CRF = corticotropin-releasing factor; EPI = epinephrine; HPA = hypothalamic-pituitary-adrenocortical; NE = norepinephrine; NPY = neuropeptide Y; PTSD = posttraumatic stress disorder; SF = Special Forces; SUDS = subjective units of distress scale; TPQ = Tridimensional Personality Questionnaire.

INTRODUCTION

Within the past decade, abundant evidence has shown that PTSD is characterized by discrete anomalies in noradrenergic neuronal reactivity (1–6) and in the HPA axis response to stress (7–9). However, the lack of prospective studies evaluating these systems in individuals *before* trauma exposure and *before* the subsequent development of PTSD prevents a determination of whether such biological abnormalities are the *result* of trauma exposure and/or the development of PTSD or whether they represent *biological traits that predispose* individuals to the development of trauma-related illness.

Although many people are exposed to trauma, only some individuals develop PTSD; most do not (10–12). It is possible that humans differ in the degree to which stress induces neurobiological perturbations of their threat response systems, which may result in a differential capacity to cope with aversive experiences. Indeed, preclinical evidence supports the hypothesis that dysregulation of neurotransmitter systems that both augment and attenuate threat responses may contribute to anxiety and stress vulnerability.

Helig et al. (12) have proposed such a relationship for the neurotransmitters NPY and CRF, both of which are released by neuron populations located in the central nucleus of the amygdala, an area critical to fear conditioning and stress responding (13, 14). Whereas CRF has "anxiogenic-like" effects, such as decreasing time on the open arms in the plus maze test (15) and increasing acoustic startle (16), NPY has been shown to exhibit anxiolytic properties in animal models of anxiety (17, 18). Alterations in either CRF or NPY release might be expected to influence the manner in which an organism responds to stress. For example, deficits in NPY might be expected to result in an increased level of anxiety and distress, whereas augmentations in NPY might result in less stress-induced distress.

Evidence from human studies supports this conceptualization. Compared with healthy subjects, individuals with combat-related PTSD reported more anxiety, had low baseline levels of NPY, and had a *blunted* NPY response to the α-2 antagonist yohimbine (19).

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These data are in agreement with preclinical data showing that chronic stress exposure may result in the development of low baseline levels of NPY as well as a blunted NPY response to subsequent stress (20). In addition, Rassmuson et al. (19) found a negative correlation between the degree of combat exposure and plasma NPY and a negative correlation between baseline NPY levels and yohimbine-induced increases in plasma 3-methoxy-4-hydroxyphenylglycol and in systolic blood pressure. Thus, it is possible that the sympathetic dysregulation repeatedly documented in PTSD subjects may be due in part to deficits in the NPY response to stress.

Additional support for the idea that an adequate NPY response may buffer the impact of stress comes from an investigation of humans experiencing uncontrollable stress during military survival school training. The stress of events during the practical exercise phase of the training produced marked changes in a number of neuroendocrine responses compared with baseline measures (21, 22). Increases in serum cortisol were robust and comparable to levels documented in individuals exposed to real-world, life-threatening events (21). Stress-induced NPY release was also marked and was significantly greater in soldiers who successfully completed extensive training to obtain membership in special units (ie, Special Forces or Green Berets) compared with soldiers assigned to conventional and elite infantry units that have high fitness and training standards (ie, Rangers and Marines). Twenty-four hours after cessation of stress exposure, NPY had returned to baseline in the SF soldiers, whereas the remaining (non-SF) soldiers exhibited a significant *depletion* of NPY (22). Furthermore, stress-induced NPY release was positively correlated with mental clarity and alertness during interrogation and tended to be negatively related to psychological symptoms of dissociation. These data provided evidence that neurobiological systems involved in responses to threat may differ significantly among individuals and that such differences might be relevant to, but not restricted to, constructs such as stress resilience and stress vulnerability. However, individual neurohormonal responses to threat are part of a larger, coordinated, and interactive system. Therefore, a clearer understanding of our previous reports might be gained by examining the ways cortisol, catecholamine, and NPY responses relate to one another and to human behavior.

In this article we expand on our previous report (22) to further explore the idea that differences in the neurobiological responses of individuals exposed to threat are significantly related to the concomitant psychological and behavioral indices and now present the catecholamine responses (NE and EPI) for the subjects of our previous report (22) who were exposed to acute uncontrollable stress. We also present the *relationships* between their NE and EPI responses and their NPY and cortisol responses to threat as well as the relationship of these neurobiological indices to both psychological symptoms and human performance exhibited under stress.

METHODS

Forty-four male, active-duty soldiers (age = 27.8 years, SD = 5; weight = 176.2 lb, SD = 8) enrolled in US Army survival school training were the subjects of this study. These 44 subjects were a subset from our larger investigation (21) for whom there was enough plasma to measure catecholamines in addition to the previously reported NPY and cortisol levels (22).

As designated by their military operational specialty, 23 subjects were SF soldiers (Green Berets) and 21 were non-SF soldiers (Rangers and Marines). Ethnicity of subjects was as follows: SF: white = 22, African American = 1; non-SF: white = 18, hispanic = 3. In the SF group there were 5 captains, 2 medics, and 16 weapons experts. None had previous combat experience. Similarly the non-SF group comprised 13 Rangers (11 Bravos), 6 Marine reconnaissance soldiers, and 2 captains. None had previous combat experience. The average number of years in the service for both groups was 7 years (SD = 2.1), and the years of education was 12 (SD = 2). The methodology used in this study has been reported in detail elsewhere (21); however, a brief summary is provided here. Before enrollment in the study, each participant completed in-processing into the Army survival training course and passed medical, psychological, and equipment criteria for participation in the course. All physical and psychological examinations were conducted 30 days before enrollment in the course. No subjects met criteria for medical or psychiatric diagnoses as determined by physical and psychological evaluation. All subjects were free of illicit substances as determined by urine toxicology screening.

Recruitment of subjects for participation in the study was conducted by the principal investigator (C.A.M.) at the US Army John F. Kennedy Special Warfare Training Center and School, Fort Bragg, North Carolina. All subjects understood that the principal investigator was not in the military and that participation in the study would have no positive or negative effect on their survival school evaluations. The study protocol was approved by the human subjects committees of Yale University School of Medicine and Womack Army Hospital, Fort Bragg. All subjects gave written, informed consent. Because of programatic restrictions that limited the number of blood draws to two, the subjects of this study (N = 44) were divided into two subgroups: those whose blood was sampled at baseline and at recovery (N = 23) and those whose blood was sampled at baseline and immediately after exposure to acute stress (N = 21). Subjects were randomly assigned to the groups.

Baseline Assessment

Baseline blood samples were collected on the second day of didactic activities and five days before the stress assessment at the survival training facilities in Fort Bragg. At baseline, morning and evening salivary hormone samples were collected using Salivette tubules (Sarstedt, Newton, NC). Subjects placed the cotton ball in their mouths, chewed until it was saturated with saliva, and spit the cotton ball and extra saliva back into the tube. Baseline psycholog-

ical measures were collected during a 30-minute classroom session after the collection of baseline plasma and salivary data. Subjects completed the Cloninger TPQ (23), a reliable, valid, self-report instrument designed to assess three personality traits: novelty seeking, harm avoidance, and reward dependence.

Stress Assessment

At the conclusion of the didactic phase of the training, soldiers entered the experiential phase of the survival course. As previously described (21), during this phase the soldiers were given, in as highly realistic manner as possible, a captivity experience in the Army's training laboratory. Each soldier was subjected to unavoidable physical and mental stress. The challenges to subjects in the training laboratory are modeled after those experienced by American captives in World War II and the Korean and Vietnam Wars. These challenges include interrogations and problem-solving dilemmas designed to test the soldiers' ability to utilize and adhere to their training and a prescribed code of conduct. Oversight and supervision of this training are conducted by an organization comprised of military and civilian scientists, the Joint Personnel Recovery Agency. This agency is responsible for ensuring safe, effective, and ethical training for military personnel. Persons interested in conducting a replication study are invited to contact the principal investigator for a point of contact at the Kennedy Special Warfare Center and School.

Biological stress samples (NE, EPI, NPY, and cortisol) were obtained from 21 soldiers immediately after exposure to their first military interrogation in the training laboratory. Before interrogation stress, subjects remained in an isolation room for several hours. After this, subjects underwent interrogations, during which they remained standing and relatively immobile. Plasma and saliva samples were collected immediately after conclusion of the interrogation. Saliva was collected immediately before the collection of blood. The cotton from the collection tube was placed in subjects' mouths and the Salivette tube was held up to subjects' mouths by members of the research team (C.A.M., G.H.) to recollect the cotton and extra saliva. All subjects were subjected to uniform food deprivation before interrogation stress. Subjects underwent uniform sleep deprivation after (not before) the collection of interrogation stress samples.

Recovery Assessment

Recovery blood samples and saliva samples were collected from subjects (N = 23) 24 hours after their release from the training laboratory. Medical staff monitored subjects during their time at the training laboratory and ensured that all subjects received water on a uniform schedule throughout the training. At release from the training laboratory, each subject was given a sack lunch containing identical contents. All were confined to the barracks compound and did not leave until completion of the recovery day debriefing. On recovery day, none participated in physical exercise and all received the same breakfast and lunch. All participated in a classroom debriefing, and none ate between lunch and the recovery assessment time point.

Plasma Catecholamine Analysis

Plasma was stored at −70°C from the time of initial collection. Plasma NE and EPI were measured using high-performance liquid chromatography. Briefly, 500 μl of plasma was extracted over alumina and analyzed by reverse-phase high-performance liquid chromatography with coulometric detection; 3,4-dihydroxybenzylamine was used as internal standard. NPY and cortisol were measured as previously reported (21, 22).

Psychological Assessment

To measure how subjects had responded psychologically and subjectively to stress in the training laboratory, a subjective units of distress scale (SUDS) was administered (0 = totally relaxed; 10 = most stress ever experienced, including war zone). After completing the SUDS, subjects also completed the CADSS, a valid, reliable, self-report instrument designed to assess dissociative experiences (24). In contrast to the instructions they were given about the SUDS (to rate the stress of the training laboratory), subjects were instructed to complete the CADSS in response to the specific experience of interrogation. The subjective scale of the CADSS consists of 19 items, each of which can be endorsed by subjects on a scale of 0 (none) to 4 (extremely). The CADSS total score was the sum of values on the individual items.

Interrogation performance was scored by the survival school instructors as part of their formal role. The scales for scoring student performance are not available for public distribution. However, to facilitate an interpretation of the data, it can be said that the scores reflect whether students were able to demonstrate, while experiencing the stress of interrogation, specific behaviors that are of interest to military interrogators. These scores are accepted by the military community as a measure of mental clarity and information processing. Within the context of this naturalistic study, the scores represent a "double-blind" assessment of the subjects' performance vis a vis hormone responding because interrogation performance was scored by the survival instructors, who were blind to the hormone and dissociation data. Interrogation performance scores were not obtained for the baseline/recovery group because of a technical error on the part of the research team. According to survival school procedures, all student data are destroyed within 5 days of the time the student completes the course. The research team was unable to obtain the data within that time frame. Performance scores were obtained on the group of subjects tested at baseline and immediately after stress.

Data Analysis

Separate repeated-measures ANOVAs using the factors "time" (baseline/recovery and baseline/stress for each hormone [NE, EPI, and cortisol]) and "group" (SF or non-SF) was performed for each of the two cohorts of subjects. Post hoc comparisons were made using least square means to adjust for the slight differences in the number of subjects for each group.

Independent t tests were used to test for differences between the respective baseline NE, EPI, and cortisol hormone values for the study subsamples (ie, N = 23 and N = 21). In addition, independent t tests were performed to compare the SUDS scores and the CADSS scores of the two groups to detect whether the groups were representative of one another.

In addition to absolute scores, change scores were calculated for NE, EPI, cortisol, and NPY. This was done by subtracting baseline from recovery values or baseline from stress values for each hormone. Previous investigators (25) have suggested that the saliva/plasma cortisol ratio (percentage of free cortisol) may provide a useful index of the amount of cortisol that is truly bioavailable. Thus, percentage of free cortisol values were calculated for each assessment time point.

Pearson correlation analyses were used to evaluate relationships between independent variables (age, weight, education, number of

years in the service, as well as the three subscores from the TPQ [novelty seeking, harm avoidance, reward dependence]) and each dependent variable. Pearson correlation analyses were also used to compare the hormone values with each another (at each of the respective sampling time points) and to evaluate the relationship between hormones and psychological indices such as dissociation (CADSS total score) and performance (interrogation performance score). Stepwise linear regression analyses were used to examine whether personality factors as assessed by the TPQ predicted the values of both the hormone variables and the psychological variables (dissociation and interrogation performance).

To detect which hormone indices best predicted the value of the dependent hormone variables (NE, EPI, NPY, and cortisol) and psychological variables (dissociation and interrogation performance), separate stepwise linear regression analyses were used. Separate analyses were performed for the two groups (baseline/recovery and baseline/stress). No performance scores were available for the baseline/recovery group; thus, analyses using the dependent variable "performance" pertain to the baseline/stress group alone.

RESULTS

Baseline Cloninger TPQ scores for novelty seeking, harm avoidance, and reward dependence were 16.5 (SD = 0.6), 5.6 (SD = 0.5), and 16.5 (SD = 0.4), respectively. No significant differences were noted between the SF and non-SF groups. No significant correlations were noted between personality scores and any of the dependent hormone or psychological variables. Nor were there any significant correlations between the TPQ scores and the independent variables of age, weight, number of years in the service, education, or military operational specialty (MOS).

Retrospective SUDS ratings of training laboratory stress indicated that all subjects found participation in survival school highly stressful (mean score ± SD = 7.6 ± 1.9). Subjective stress ratings did not differ significantly between SF and general troop soldiers (7.4 ± 2.2 and 7.8 ± 1.8, respectively), nor did stress ratings differ significantly between the two test groups (baseline/recovery and baseline/stress, 7.9 ± 1.3 and 7.6 ± 2.6, respectively). Significant differences in CADSS scores were observed between SF and non-SF subjects in each of the test groups (baseline/recovery: 13.5 ± 10.2 vs. 23 ± 16.1, $t = 2.3$, $p < .025$; baseline/stress: 9.4 ± 9.8 vs. 21.5 ± 15.5, $t = 2.2$, $p < .03$). No significant differences were observed in interrogation performance scores between SF and general troop soldiers. The mean interrogation performance score was 10.3 (±2.6). Performance scores are rated on a scale with a maximum possible score of 15. A higher score is indicative of better performance.

No significant relationships were found between the independent variables of age or weight and the dependent variables at any of the sampling time points. Therefore, age and weight were removed from subsequent analyses.

Effect of Stress on Hormones

Baseline/recovery group. In the group of subjects tested at baseline and at recovery, a repeated-measures ANOVA conducted on the NE values with factors time (baseline and recovery) and group (SF and non-SF) revealed a statistically significant within-subject and between-subjects effect of time ($F(1,21) = 47.5$, $p < .0001$; $F(1,21) = 103.5$, $p < .0001$, respectively) but not group. No significant time-by-group interaction was observed. Similarly, a repeated-measures ANOVA conducted on the EPI values with factors time (baseline and recovery) and group (SF and non-SF) revealed statistically significant within-subject and between-subjects effects for time only ($F(1,21) = 4.2$, $p < .05$; $F(1,21) = 66.1$, $p < .0001$, respectively). Inspection of the data revealed a trend for a significant, between-subjects effect of group ($F(1,21) = 2.2$, $p < .1$). Post hoc t tests comparing recovery EPI values of the SF and non-SF groups revealed a similar trend toward significance ($df = 21$, $t = 2.0$, $p < .06$). No time-by-group interaction was observed.

Baseline/stress group. In the group of subjects tested at baseline and immediately after interrogation stress, a repeated-measures ANOVA of the NE values revealed significant within-subject effects for time ($F(1,17) = 150.7$, $p < .0001$) and a significant group-by-time interaction ($F(1,17) = 4.3$, $p < .05$). Post hoc t tests indicated that this group-by-time interaction was due to the fact (as shown in Figure 1, *A*) that NE values of SF soldiers were significantly greater than those of non-SF soldiers in response to interrogation stress (1529.9 ± 483 vs. 1089.6 ± 244, $df = 18$, $t = 2.6$, $p < .02$). ANOVA also revealed significant between-subjects effects for time ($F(1,17) = 261.4$, $p < .0001$) and group ($F(1,17) = 5.8$, $p < .03$).

Plasma epinephrine was also increased (Figure 1, *B*) by exposure to acute stress. A repeated-measures ANOVA for the EPI values, using the factors time (baseline and stress) and group (SF and non-SF), revealed a significant within-subject effect of time ($F(1,17) = 29.9$, $p < .0001$). No time-by-group interaction was observed. A significant between-subjects effect was also observed for time ($F(1,17) = 113.6$, $p < .0001$) but not group.

Consistent with our previous report (21), a repeated-measures ANOVA using time (baseline and recovery) and group (SF and non-SF) revealed significant within-subject effects of time ($F(1,21) = 37.6$, $p < .0001$) and time by group ($F(1,21) = 5.9$, $p < .02$) for serum cortisol. This was due to the fact that the recovery day values of cortisol as well as the amount of change in cortisol (from baseline to recovery) was *smaller* in the SF than in the non-SF group. However, a repeated-

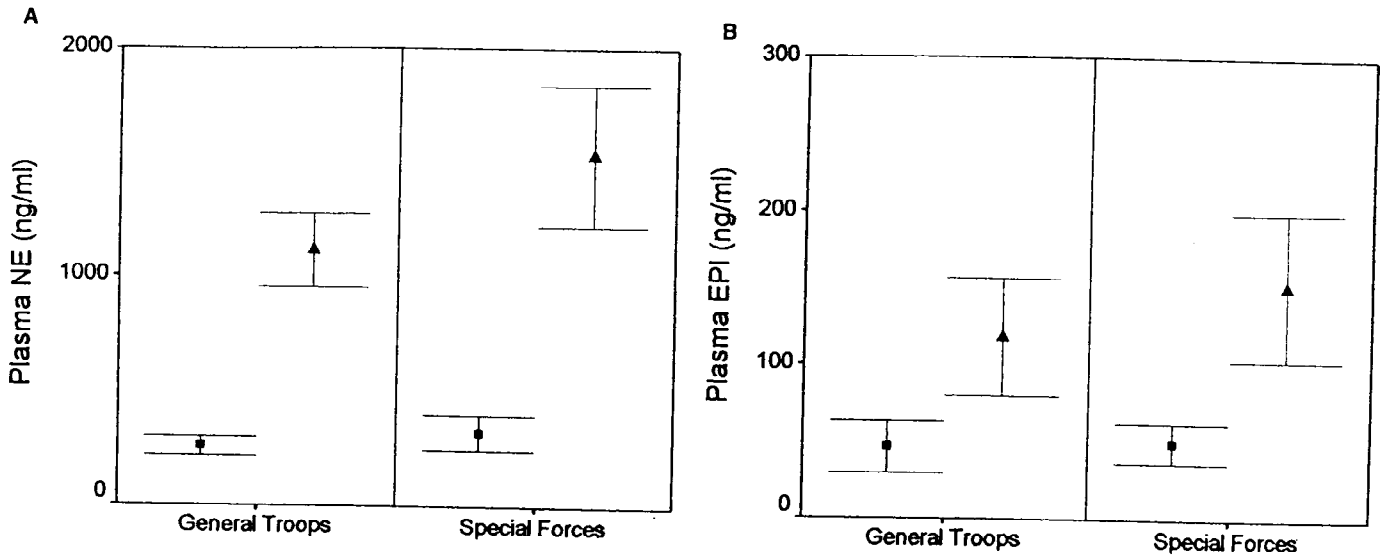

Fig. 1. (*A*) NE at baseline (■) and during stress (▲). Acute stress significantly increased NE in all subjects. NE release was significantly greater in SF soldiers ($p < .05$). (*B*) EPI at baseline (■) and during stress (▲). Exposure to stress significantly increased EPI ($p < .0001$). No significant group differences were observed.

measures ANOVA of cortisol values using time (baseline and stress) and group (SF and non-SF) revealed a significant within-subject effect of time only ($F(1,19) = 163.2$, $p < .0001$). No significant group-by-time interaction was observed ($p < .4$).

Relationships Between Hormones

In the baseline/recovery group there were significant correlations between NE and EPI at baseline ($r = 0.58$, $p < .003$) and at recovery ($r = 0.58$, $p < .003$). There was a trend for a significant correlation between EPI and NPY at recovery ($r = 0.41$, $p < .06$). In the baseline/stress group, significant correlations were observed between NPY and cortisol ($r = 0.67$, $p < .001$), NPY and NE ($r = 0.55$, $p < .01$), and NPY and EPI ($r = 0.55$, $p < .01$).

Significant correlations were also observed in the baseline/stress group between the *change* in NPY and change in NE ($r = 0.53$, $p < .02$) (Figure 2) and between the change in NPY and change in cortisol ($r = 0.73$, $p < .0001$) (Figure 3). In addition, a significant positive correlation was observed between the *percentage* of free cortisol and NPY ($r = 0.5$, $p < .04$).

Relationships Between Hormones, Dissociation Scores, and Performance

As illustrated in Figure 4, significant negative correlations were observed between dissociation scores and percentage of free cortisol after interrogation stress ($r = -0.49$, $p < .04$) as well as between dissociation scores and interrogation performance scores ($r = -0.69$, $p < .0001$). In contrast, percentage of free cortisol at recovery was significantly and positively correlated with dissociation scores ($r = 0.46$, $p < .04$). Significant positive correlations were observed between interrogation performance scores and percentage of free cortisol in response to interrogation stress ($r = 0.45$, $p < .04$) (Figure 5) as well as between interrogation performance scores and NPY after interrogation stress ($r = 0.58$, $p < .006$).

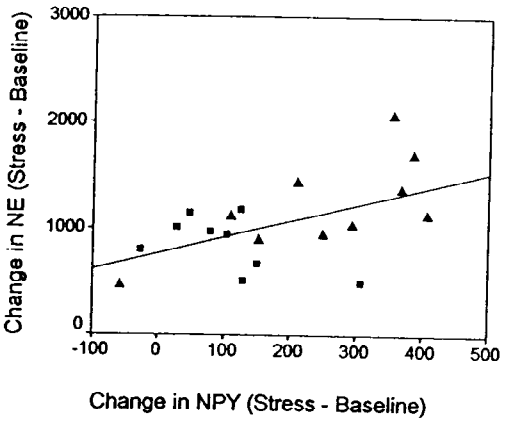

Fig. 2. NPY and NE during stress. Correlation between change in NPY and change in NE ($r = 0.53$, $p < .02$). ■ = general troops; ▲ = Special Forces. Total population $R^2 = 0.2830$ (■ = 0.00; ▲ = 1.00).

Stepwise Linear Regression Analyses

Separate stepwise linear regression analyses were conducted on NE, NPY, and cortisol during stress (Table 1) to determine the degree to which each hormone

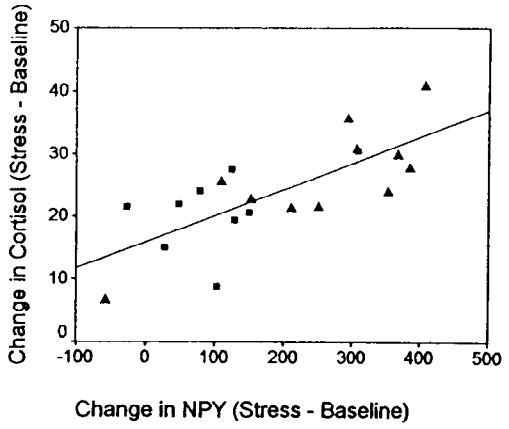

Fig. 3. Cortisol and NPY during stress. Correlation between change in NPY and change in cortisol ($r = 0.73$, $p < .001$). ■ = general troops; ▲ = Special Forces. Total population $R^2 = 0.5387$ (■ = 0.00; ▲ = 1.00).

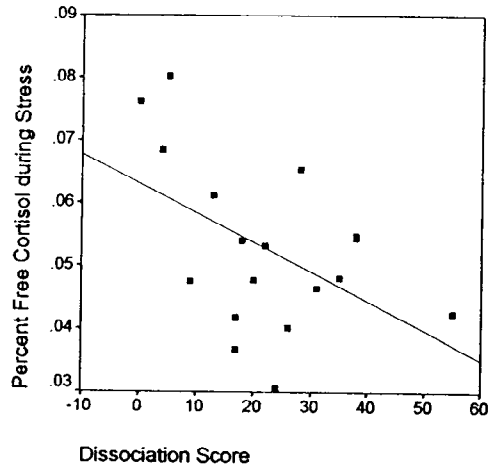

Fig. 4. Cortisol and dissociation during stress. $R^2 = 0.2379$.

accounted for the variance of other hormones during stress. Stepwise linear regression analyses were also conducted using percentage of free cortisol during stress and NPY during stress as the independent variables and CADSS dissociation scores as the dependent variable (Table 2). The model was significant ($F(1,15) = 5.6$, $p < .032$) and accounted for 22% of the variance in dissociation scores (adjusted $R^2 = 0.22$). The standardized coefficient (β) for percentage of free cortisol during stress was -0.52 ($t = -2.4$, $p < .032$). NPY during stress did not make a significant contribution to the variance of dissociation. Similar stepwise linear regression analyses were conducted on the data from the baseline/recovery group, this time using percentage of free cortisol at recovery and NPY at recovery as the independent variables and CADSS dissociation scores as the dependent variable. The model was significant ($F(1,18) = 4.9$, $p < .04$) and accounted for 18% of the variance in CADSS scores (adjusted $R^2 = 0.18$).

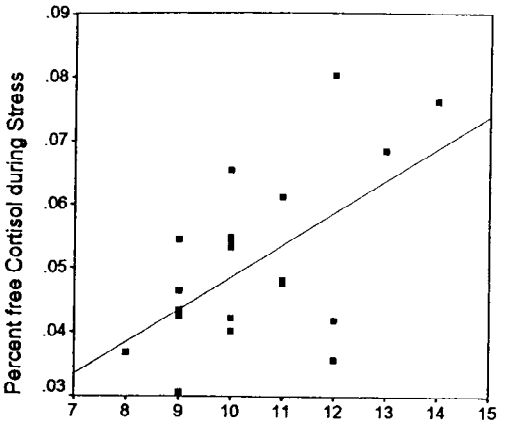

Fig. 5. Cortisol and performance during stress. $R^2 = 0.3346$.

TABLE 1. Stepwise Linear Regression Analyses Examining Dependent Variables NE, NPY, and Cortisol During Stress

Variable	Adjusted R^2	Variance (%)	β	p
NE				
NPY during stress	0.30	30	0.55	<.01
NPY				
Cortisol during stress	0.42	42	0.55	<.005
Cortisol and NE during stress	0.51	51	0.36	<.05
Cortisol				
NPY during stress	0.42	42	0.67	<.001

Percentage of free cortisol at recovery had a β value of 0.48 ($t = 2.2$, $p < .04$). NPY recovery values did not make a significant contribution.

Stepwise linear regression analyses using interrogation performance scores as the dependent variable and percentage of free cortisol during stress and NPY during stress as the independent variables indicated that the model was significant ($F(1,18) = 9.4$, $p < .007$). The model accounted for 31% of the variance (adjusted $R^2 = 0.31$). Percentage of free cortisol during stress had a β value of 0.59 ($t = 3$, $p < .007$). NPY did not make a significant contribution.

DISCUSSION

Healthy human subjects exhibited significant differences in the degree to which exposure to uncontrollable stress produced perturbations in neuroendocrine systems sensitive to threat. These differential responses were significantly linked to how well subjects reacted psychologically and behaviorally in response to the stress of survival school.

Exposure to acute, uncontrollable stress resulted in significant, robust increases in plasma catecholamines

TABLE 2. Stepwise Linear Regression Analyses Examining Dependent Variables Dissociation and Performance

Variable	Adjusted R^2	Variance (%)	β	p
Dissociation				
Percentage of free cortisol during stress	0.22	22	−0.52	<.03
Percentage of free cortisol at recovery	0.18	18	0.48	<.04
Performance				
Percentage of free cortisol during stress	0.31	31	0.59	<.007

and cortisol across all subjects. The findings of this study not only support a large body of work in humans demonstrating that NE, EPI, and cortisol are increased in association with emotional distress, but also add to that literature in several ways. First, the findings establish that acute, uncontrollable psychological stress is capable of inducing sympathetic and adrenal medullary activation of a magnitude comparable to real-world, potentially life-threatening experiences (26, 27). Second, they provide information concerning concurrent neurohormonal and psychological indices in response to acute stress. For example, significant correlations were observed between catecholamines and NPY during stress and also between NPY and cortisol indices during stress. Third, these data show that the percentage of free cortisol explained a significant amount of the variance in psychological symptoms of dissociation and in human performance. To our knowledge, this is the first naturalistic study linking neuroendocrine responses to both psychological symptoms of dissociation and objectively assessed behavioral performance.

For the group as a whole, interrogation stress resulted in mean plasma NE and EPI values of 1309.8 pg/ml (SD = 436) and 133.2 pg/ml (SD = 67), respectively. These compare with NE and EPI values reported for novice parachutists (900 and 400 pg/ml, respectively) (28) or intubated patients undergoing endobronchial suctioning (1673 and 368 pg/ml, respectively) (29). Similarly, the magnitude of stress-induced serum cortisol (33.6 μg/dl) is comparable to that seen in novice parachutists (24 μg/dl) (28) or patients undergoing open-heart surgery (36.8 μg/dl) (27). Together these data indicate that the US Army survival school training laboratory is a valid model for prospectively investigating uncontrollable or "threat-to-life" stress in humans.

Consistent with our previous report on plasma NPY responses to acute stress (22), plasma NE during such stress was significantly greater in SF than in non-SF soldiers. Stress-induced EPI release did not differ between these two groups. These data are compatible with preclinical evidence that that NE and NPY are colocalized and are coreleased under conditions of high stress (30, 31). The fact that NE, but not EPI, was differentially increased in SF soldiers suggests that the source of the previously reported stress-induced increases in plasma NPY (22) may be cells located in sympathetic ganglia.

Because most clinical studies investigating catecholamine responses to acute stress have been concerned with the acute phases of sympathetic adrenal activity and have limited assessment time points to minutes or hours after stress exposure, few data exist regarding the biological aftermath of stress exposure in healthy human subjects. The current study shows that NE may remain significantly elevated for at least up to 24 hours after stress, whereas EPI may be reduced. These findings are consistent with recovery hormone data reported in primates exposed to unavoidable stress (32) and may be relevant to contemporary questions about poststress learning, memory formation, and poststress illness. Given the large body of preclinical evidence for the role of catecholamine functioning in the consolidation of memory (33–35), it is likely that many individuals (those with higher NE) are primed for optimal memory encoding on recovery day debriefings. It is also possible that some individuals (those who are depleted in NPY and in EPI) may be less equipped in poststress memory consolidation (33, 34).

Peripheral change in cortisol and peripheral change in NPY were highly correlated. This resembles the hypothesized relationship between central CRF and NPY responses within the amygdala (12). Clearly the relationship between peripheral and central NPY/cortisol dynamics is not well understood; however, the data are noteworthy given the preclinical evidence of a reciprocal regulation of CRF and NPY in areas of the brain critically involved in the appraisal of and response to threat, such as the amygdala and periaqueductal gray (36, 37). Activation of brain CRF systems may lead to increases in central NE release along with NPY release (38). Numerous animal studies have demonstrated the capacity for NPY to inhibit the release of NE from peripheral and central noradrenergic neurons and to inhibit the firing of locus ceruleus neurons involved in the arousal response (31). In addition, NPY has been shown to exert antianxiety effects via Y1 receptors in the central nucleus of the amygdala and in periaqueductal gray (17). Furthermore, antisense inhi-

bition of NPY Y1 receptor expression blocks this anxiolytic-like action of NPY. These CRF/NPY interactions are highly relevant to human stress responses in that abnormalities of both CRF and NPY have been noted in individuals suffering from posttraumatic stress, anxiety, and symptoms of dissociation (8, 19).

The present data show that individuals differ in peripheral cortisol/NPY relationships and that such differences have relevance to the way individuals respond both psychologically and behaviorally to stress. One might speculate that differences in central relationships between CRF and NPY may also exist due to the fact that both cortisol and NPY (22) were significantly related to symptoms of dissociation, which is presumably a central nervous system process. Theoretically the central CRF and NPY relationship might be further evaluated in humans by assessing serial cerebrospinal fluid samples during or in response to a pharmacologic challenge stimulus capable of eliciting central release of CRF, NE, and NPY as well as psychological symptoms of dissociation. The data from the current investigation do not provide definitive evidence for central differences in CRF and NPY between SF and non-SF soldiers. However, this may be fruitful avenue of future research.

Neither NE nor EPI was significantly associated with either interrogation performance or psychological symptoms of dissociation. Both NPY and cortisol were significantly related to these variables. Preclinical data show that intracerebroventricular infusion of NPY in rats exposed to intruder stress results in decreased social stress as measured by significant reductions in blood pressure, heart rate, and activity (39). NPY has also been shown to influence mammalian behavior in a manner comparable to that observed with established anxiolytic compounds (such as chlordiazepoxide) (40). Thus, it is possible that stress-induced release of CRF (and cortisol) elicited a corresponding release of NPY, which, because of its antistress properties, resulted in the negative correlations between symptoms of dissociation and both NPY and cortisol.

The saliva/blood ratio of cortisol best accounted for the variance in dissociation and in interrogation performance. Previous reports have demonstrated that individuals differ significantly in the degree to which circulating cortisol may be bound to corticotropin-binding globulin under both nonstressful and stressful conditions (21, 25). The present data indicate that the percentage of free glucocorticoid is associated with enhanced performance. Whether this effect is mediated through a central CRF/glucocorticoid-enhanced NPY release or through increased adrenal activation and release of androgens (such as dehydroepiandrosterone; Ref. 41) is unknown at this time.

Although a negative correlation was noted during stress, at recovery there was a positive correlation between percentage of free cortisol and dissociation. One explanation may be that certain individuals, as they encounter stress, reach a threshold at which they are unable to counterregulate their stress response with an adequate release of NPY. As the stress continues to increase, they are unable to tolerate the stress and dissociate (or "disconnect") from the environment. This disengagement, like the psychological defense of denial, may shield them psychologically from threat and result in decreased HPA axis activation and lower cortisol (42). In contrast, individuals with an increased capacity for NPY release remain engaged in the stressful situation. They continue to release cortisol but do not experience as much dissociation due to increases in NPY. This would result in a negative relationship between the percentage of free cortisol and dissociation during stress. The *positive* correlation between percentage of free cortisol and dissociation noted at recovery may reflect the presence of a threshold below which NPY is not released. An alternative explanation for this is that individuals for whom interrogation stress was intolerable (and who experienced more dissociation) remain more "activated" at recovery and exhibit higher percentages of free cortisol, resulting in a positive relationship between the percentage of free cortisol at recovery and dissociation. Future neurobiological challenge studies with a greater number of assessment time points for these variables might clarify this issue. It is also possible that pretreatment with a cortisol synthesis–blocking agent (ie, ketoconazole) might help clarify whether the symptoms of dissociation are mediated through central CRF activity and/or through peripheral cortisol activity at the hippocampus (43).

SF soldiers demonstrated a greater capacity for NPY release under stress, a rapid return to baseline levels of NPY at recovery, and enhanced NE release under stress. They exhibited overall less HPA axis activation in response to stress (as measured by baseline/recovery differences in cortisol). This pattern is consistent with the model of stress "toughening" or "stress hardiness" put forward by Deinstbier (44). As demonstrated in superior athletes, stress hardiness has been characterized as a rapid and efficient response to a stressor followed by a quick return to baseline or resting levels. In this article we invoke a "stress hardiness/stress toughening" hypothesis regarding the subjects because this use of the term is in keeping with the way the term has been used in describing the hormone responses of animals considered "toughened" by stress exposure. This is one of many possible ways one might characterize stress hardiness, yet it is useful here be-

cause of its basis in animal literature. This model underlies the hypothesis that some subjects in our study would be less likely to develop stress-related illnesses such as PTSD than individuals lacking the "stress-toughened" neuroendocrine response to threat.

SF and non-SF subjects did not differ significantly in their interrogation performance scores. It is possible that the relatively small sample size prevented a detection of group differences. However, the lack of a group difference is not a commentary on the stress hardiness of subjects, because the performance scores were not designed to measure toughness but rather to reflect the soldiers' ability to apply what they had learned during the survival course. These preliminary data simply address the possible relationships among psychological, endocrine, and performance indices.

To be in Special Forces, an individual must have successfully completed the Special Forces Selection and Assessment Course, followed by the Special Forces Qualification Course, both of which involve a high level of challenge to physical endurance, academic abilities, and stress tolerance. It is possible that the neurobiological differences between SF and non-SF soldiers develop over time during this training sequence. Alternatively, such differences may be due to personality traits, level of physical fitness, time in the service, or a result of the specialized training administered to individuals once they join the SF community. Investigations of the selection and assessment course are currently testing these hypotheses.

The present data do not rule out the role of personality traits in subjects' responses to stress. The restricted range noted in the TPQ responses may be an artifact created by the demand characteristics at the time of assessment. Soldiers are familiar with psychological testing and often worry that their responses might negatively affect their status in training. Some evidence for this idea is found in the way subjects completed questionnaires at the completion of training. Many CADSS items could be perceived as "abnormal," yet they were frequently endorsed. Thus, once subjects have completed the course, they seem more forthcoming. Until this is clarified, it is premature to conclude that personality traits do not contribute to neuroendocrine or psychological responses to threat. A more fruitful method of assessment might involve collection of personality data well before participation in survival school training.

There are limitations to this study. First, it was not possible to obtain blood samples from all subjects at three time points. This reduced our sample size and necessitated certain inferences about the two groups (baseline/stress and baseline/recovery). However, these groups did not differ at baseline on any of the variables assessed, nor did they differ in their relative composition of subjects; thus, it is reasonable to consider them representative of one another.

Second, peripheral measures were used to assess neuroendocrine responses to stress. Although peripheral response patterns may appear strikingly similar to those found centrally, the nature of the relationship between peripheral and central measures of NPY, cortisol (CRF), and NE are the subject of much investigation at this time.

A third limitation is the relative homogeneity of military groups. It is possible that the high levels of variance in psychological and performance data explained by hormones is reflective of this homogeneity. Participants in survival training are individuals who have decided to engage in dangerous work. It is likely that their stress responses may not represent those of soldiers less inclined to hazardous military duty. However, because certain types of civilian work (eg, firefighting and law enforcement) expose people to life-threatening circumstances, these data have relevance outside the military community and for current research in PTSD.

In this report the training laboratory stress is labeled "uncontrollable." Subjects experienced circumstances in the laboratory over which they had no physical or verbal control. It was impossible for subjects to exert an influence over the stress through physical or verbal courses of action. Participants are expected to apply what they have been taught to cope with circumstances that are beyond their direct control. A student may "take control" of the situation by explicitly stating that he no longer wishes to continue training. This decision is considerable and is likely to result in disqualification from special operations missions and in reduced career opportunities. The degree to which this type of uncontrollable stress overlaps with the construct applied to preclinical investigations awaits further clarification.

In summary, the present data provide robust evidence that individual variation in neuroendocrine responses may explain some psychological and behavioral responses to acute stress. These individual differences exist *before* trauma exposure and may be used to test constructs of stress hardiness and stress vulnerability in humans. This type of distinction may promote the exploration of a "selective fitness" hypothesis in the development of PTSD. Conversely, it may be demonstrated that enhanced stress resilience can be developed over the course of training, providing support to the ideas underpinning stress inoculation theory.

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